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ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of β-GLUCURONIDASE
(EC 3.2.1.31)
from E. coli

PRINCIPLE:

PheP-Gluc + H_2O $\xrightarrow{\beta\text{-Glucuronidase}}$ D-Glucuronate + Phenolphthalein

Abbreviation used:
PheP-Gluc = Phenolphthalein Glucuronide

CONDITIONS: T = 37°C, pH = 6.8, A_{540nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 75 mM Potassium Phosphate Buffer, with 1.0% (w/v) Bovine Serum Albumin, pH 6.8 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379 and Albumin, Bovine, Sigma Prod. No. A-4503. Adjust to pH 6.8 at 37°C with 1 M KOH.)

B. 3.0 mM Phenolphthalein Glucuronide Substrate Solution (PheP-Gluc)
(Prepare 10 ml in deionized water using Phenolphthalein Glucuronic Acid, Free Acid, Sigma Prod. No. P-0501.)

C. 200 mM Glycine Buffer Solution, pH 10.4.
(Use Glycine Buffer Solution, Sigma Stock No. 105-2, or prepare 100 ml in deionized water using Glycine Free Base, Sigma Prod. No. G-7126. Adjust to pH 10.4 at 37°C with 1 M NaOH.)

D. β-Glucuronidase Enzyme Solution
(Immediately before use, prepare a solution containing 400 - 800 units/ml of β-Glucuronidase in cold Reagent A.)

E. 95% (v/v) Ethanol
(Prepare 20 ml in deionized water using 200 Proof USP Ethyl Alcohol, Quantum Chemical Corporation.)

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REAGENTS: (continued)

F. 0.05% (w/v) Phenolphthalein Standard Solution (Std Soln)
(Prepare 5 ml by dissolving 2.5 mg of Phenolphthalein, Sigma Prod. No. P-9750 in 5 ml of Reagent E or use Phenolphthalein Standard Solution, Sigma Stock No. 105-1.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable containers:

	Test	Blank
Deionized Water	0.65	0.65
Reagent A (Buffer)	0.50	0.50
Reagent B (PheP-Gluc)	0.25	0.25

Mix by inversion and equilibrate to 37°C. Then add:

Reagent D (Enzyme Solution)	0.10	------

Mix by inversion and incubate at 37°C for exactly 30 minutes. Then add:

Reagent C (Glycine Buffer)	5.00	5.00
Reagent D (Enzyme Solution)	------	0.10

Immediately mix by inversion. Transfer the solutions to suitable cuvettes and record the A_{540nm} for both the Test and Blank using a suitable spectrophotometer.

COLORIMETRIC ASSAY:

Standard Curve:

Prepare a standard curve by pipetting (in milliliters) the following reagents into suitable containers:

	Std 1	Std 2	Std 3	Std 4	Std 5	Blank
Deionized Water	0.65	0.65	0.65	0.65	0.65	0.65
Reagent A (Buffer)	0.50	0.50	0.50	0.50	0.50	0.50
Reagent B (PheP-Gluc)	0.25	0.25	0.25	0.25	0.25	0.25
Reagent F (Std Soln)	0.02	0.03	0.05	0.07	0.10	----
Reagent E (Ethanol)	0.08	0.07	0.05	0.03	----	0.10
Reagent C (Glycine Buffer)	5.00	5.00	5.00	5.00	5.00	5.00

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COLORIMETRIC ASSAY: (continued)

Mix by inversion and transfer the standards to suitable cuvettes. Record the A_{540nm} for each standard using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

ΔA_{540} Standard = A_{540} Standard - A_{540} Standard blank

Prepare a standard curve by plotting the ΔA_{540} for the Standard vs micrograms of Phenolphthalein.

Sample Determination:

ΔA_{540} Sample = A_{540} Sample - A_{540} Sample blank

Determine the total micrograms of phenolphthalein liberated using the Standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{g phenolphthalein released})(2)(df)}{0.1}$$

2 = Time correction of assay (Unit Definition = 1 hour)
df = Dilution factor
0.1 = Volume (in milliliter) of enzyme used

$$\text{Units/g solid} = \frac{\text{units/ml enzyme}}{\text{g solid/ml enzyme}}$$

$$\text{Units/g protein} = \frac{\text{units/ml enzyme}}{\text{g protein/ml enzyme}}$$

UNIT DEFINITION:

One Sigma or modified "Fishman" unit will liberate 1.0 µg of phenolphthalein from phenolphthalein glucuronide per hour at pH 6.8 at 37°C.

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FINAL ASSAY CONCENTRATION:

In a 1.50 ml reaction mix, the final concentrations are 30 mM potassium phosphate, 0.50 mM phenolphthalein glucuronic acid, and 40 - 80 units β-glucuronidase.

REFERENCES:

Fishman, W.H. and Bernfeld, P. (1955) *Methods in Enzymology*, Volume I, 262-269

Combie, J., Blake, J.W., Nugent, T.E., and Tobin, T. (1982) *Clin. Chem.* **28**, 83-86

Fishman, W.H. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed) 2nd ed., Volume II, 930-932, Academic Press, New York, NY

NOTES:

1. This assay is based on the cited references.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.